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Product datasheet for AM26619AF-N

BID (61-118) Mouse Monoclonal Antibody [Clone ID: 5C9]

Product data:

Product Type:	Primary Antibodies
Clone Name:	5C9
Applications:	WB
Recommended Dilution:	Western blot: 1-5 µg/ml for chemiluminescence detection system. For details see protocol below.
Reactivity:	Human
Host:	Mouse
Isotype:	IgG1
Clonality:	Monoclonal
Immunogen:	Recombinant human BID (61-118 aa)
Specificity:	This antibody reacts with BID.
Formulation:	PBS containing 50% glycerol, pH 7.2. No preservative is contained. State: Azide Free State: Liquid Ig fraction
Concentration:	lot specific
Purification:	Protein A agarose
Conjugation:	Unconjugated
Storage:	Upon receipt, store undiluted (in aliquots) at -20°C. Avoid repeated freezing and thawing.
Stability:	Shelf life: One year from despatch.
Gene Name:	BH3 interacting domain death agonist
Database Link:	Entrez Gene 637 Human P55957

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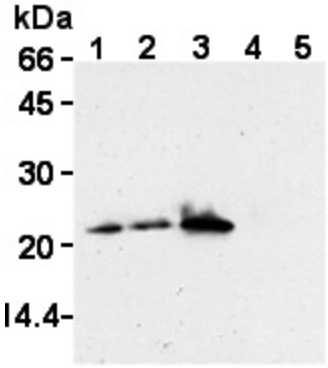
Background: Apoptosis is a major form of cell death characterized by several morphological features that include chromatin condensation and fragmentation, cell membrane blebbing, and formation of apoptotic bodies. BID is an apoptosis promoting protein, which possesses only BH3 domain that is a common structure of Bcl-2 family members. Once full length BID (p22) is cleaved with caspases, cleaved BID (p 15) induces a conformational change and oligomerization of a protein named BAK on mitochondrial membrane. It is thought that oligomerized BAK contribute to forming pore s that release cytochrome c into cytosol.

Synonyms: p22 BID

Note: This product was originally produced by MBL International.

Protocol: SDS-PAGE & Western Blotting
1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mM Tris-HCl, pH 7.2, 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol) containing appropriate protease inhibitors. Incubate it at 4 o C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4 o C and transfer the supernatant to another tube. Measure the protein concentration of the supernatant and add the cold Lysis buffer to make 8 mg/mL solution.
3) Mix the sample with equal volume of Laemmli's sample buffer.
4) Boil the samples for 2 minutes and centrifuge. Load 10 µ L of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
5) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm 2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacture's manual for precise transfer procedure.
6) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4 o C.
7) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggest in the APPLICATIONS for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 6 times).
9) Incubate the membrane with the 1:10,000 HRP-conjugated anti-mouse IgG diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
10) Wash the membrane with PBS-T (5 minutes x 6 times).
11) Wipe excess buffer on the membrane, then incubate it with appropriate chemilumi nescence reagent for 1 minute. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
12) Expose to an X-ray film in a dark room for 5 minutes. Develop the film as usual. The condition for exposure and development may vary.
(Positive controls for Western blotting; ZR-75-1, U937, HL-60)

Product images:



Western blot analysis of human BID expression in ZR-75-1 (1), U937 (2), HL-60 (3), WR19L (4) and PC12 (5) using AM26619AF-N.